



Assessing mitochondrial and cellular bioenergetics in human pluripotent stem cells



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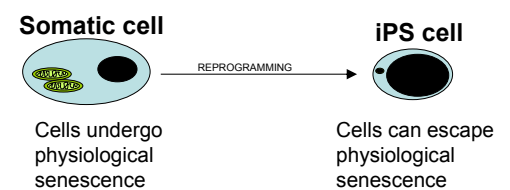
Introduction

Human somatic cells have been successfully reprogrammed to a state similar to human embryonic stem cells (hESCs) [1,2,3]. These reprogrammed cells, named induced pluripotent stem cells (iPSCs), can escape cellular senescence and appear almost identical to hESCs. Indeed, they share the same cardinal features of self-renewal (i.e. the ability to proliferate indefinitely) and pluripotency (i.e. the potential to generate all cell types of the body) [4]. Thus, iPSCs hold great promise for regenerative medicine, including autologous transplantation and *in vitro* modelling of complex disorders, such as aging and neurodegeneration.

Objectives

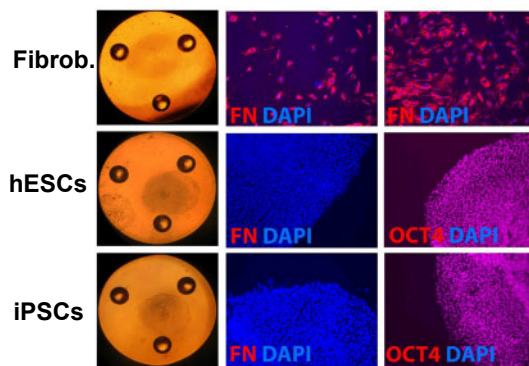
Little is known about the bioenergetic profile of human pluripotent stem cells. Mitochondria undergo specific changes during development and cellular and organismal aging [5,6,7]. We recently demonstrated that somatic mitochondria within iPSCs are capable of acquiring hESCs-like properties [6]. Both hESCs and iPSCs displayed low mitochondrial number, immature mitochondria with underdeveloped cristae, and low levels of oxidative stress-mediated intra-cellular damage [8,9]. Here, we aimed to address whether the overall bioenergetic profile of pluripotent stem cells can be assayed using a live quantitative measurement.

iPSCs appear almost identical to hESCs and share the same cardinal features of self-renewal and pluripotency



Results

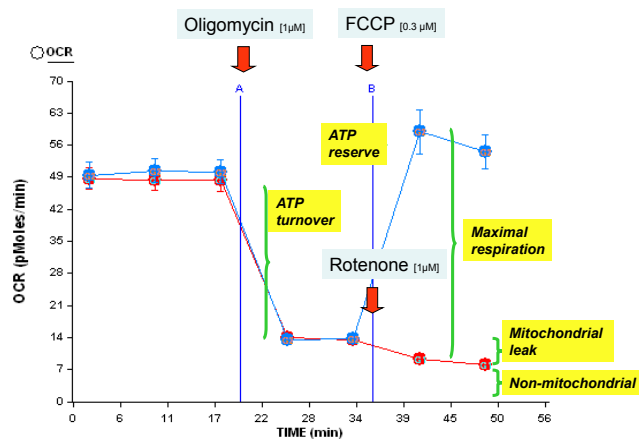
Mitochondrial and cellular bioenergetics were assessed using Seahorse XF96 (extracellular flux) analyzer (Seahorse Bioscience). The instrument utilizes special 96 well plates and allows the simultaneous quantification of mitochondrial respiration rate (oxygen consumption rate, OCR) and lactic acid generation (extracellular acidification rate, ECAR).



Growth experiments were carried out to demonstrate whether hESCs and iPSCs could retain the expression of key pluripotency-associated marker genes when grown on matrigel-coated XF96 well plates. Two human neonatal foreskin fibroblasts (HFF1 and BJ), two hESCs (H1 and H9), and two iPSCs (generated from HFF1 and BJ fibroblasts) were utilized. The cells were fixed and stained for fibroblast-specific marker (Fibronectin, FN) and pluripotency-associated marker OCT4. Nuclei were counter-stained with DAPI.

The results demonstrated that human fibroblasts maintained the expression of FN when grown on XF96 well plates. Both hESCs and iPSCs also maintained their properties: the colonies exhibited sharp edges and appeared negative for FN and positive for OCT4.

For neonatal foreskin fibroblasts, 10,000 fibroblasts were plated in each well 18h before the experiment. For hESCs and iPSCs, cells were mechanically split and grown in feeder-conditioned media for 72h before the analysis. Unbuffered media was prepared by dissolving DMEM Base (Sigma #D5030) in 1L dH₂O plus 1.85g NaCl (Sigma #S3014) and 15mg phenol red (Sigma #P-5530); 20ml were then removed from the media, and 10ml of glutamine and 10ml of 100mM sodium pyruvate were added (both from Invitrogen), together with 25mM glucose. Before the experiments, media was pre-warmed to 37°C, set to a pH of 7.4, and finally filter sterilized. Assays were initiated by removing the growth medium and replacing it with unbuffered media. The cells were incubated at 37°C for 30 min to allow media temperature and pH to reach equilibrium before starting the baseline measurement.



Three mitochondrial inhibitors (all from Sigma) were used in succession to monitor mitochondrial properties of somatic cell and pluripotent stem cells:

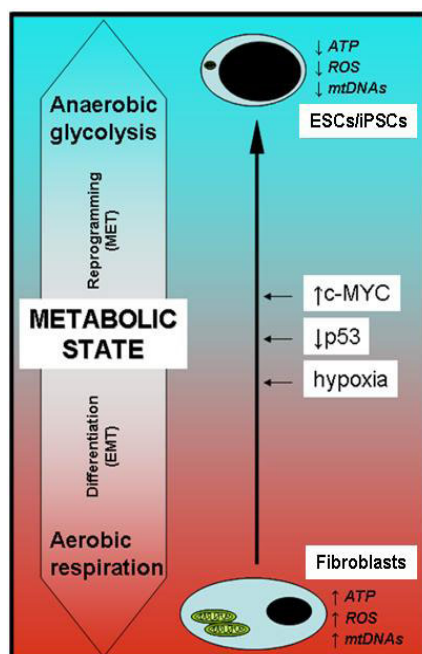
- 1) 1µM Oligomycin (time point A), which blocks Complex V and thus inhibits OXPHOS,
- 2) 0.3 µM FCCP (time point B), which is uncoupling agent leading to collapse of the mitochondrial membrane potential and to consumption of energy and oxygen without the generation of ATP,
- 3) 1µM Rotenone (time point B), a Complex I inhibitor which blocks mitochondrial respiration, hence enabling the calculations of both mitochondrial and non-mitochondrial respiratory fractions.

Conclusion

• Seahorse XF96 appeared as a reliable tool for live quantification of cellular and mitochondrial bioenergetics in human pluripotent stem cells.

• Cellular reprogramming induces an extensive remodeling of somatic stem cells [8,9]. Thus, it would be interesting to investigate whether the bioenergetic profile of somatic cells can also undergo modification upon reprogramming to pluripotency.

• Overall, monitoring the bioenergetic profile could represent a novel and relevant aspect for iPSCs-ESCs comparison and might shed some light on the mechanisms required for acquiring, maintaining and exiting a self-renewal pluripotent state in human cells.



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References

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